

methanol extracts were boiled to near dryness and redissolved in distilled water. They were then freeze-dried. Various concentrations of the aqueous extracts and methanol extracts were made in appropriate media and tested. The plants with anti-infective activities are listed in table 1.

The following experiments are presented to indicate salient features of the invention by way of examples.

1. Inhibitory effects of plant extracts against cytopathicity of HSV, HCMV, PV, MV, and YFV.

Cell death or cytopathicity is characteristic of HSV, HCMV, PV, MV, and YFV when these viruses are used to infect cells in vitro. Inhibition of viral induced cell death will therefore be an indication of antiviral activities of the plant extracts.

The procedure used was similar to previously published ones (Ayisi et al, 1980, 1983, 1985). Monolayer Vero cells in 96-well microtitre plates were infected with 100 TCID<sub>50</sub>/well of HSV, PV (types 1, 2, and 3), MV, or YFV. After 40 mins or 1 hr adsorption, unadsorbed virus was removed, the monolayers washed twice, and plant extracts at various concentrations of 2-fold dilutions added. After 3 days of incubation at 37°C in 5% CO<sub>2</sub> humidified incubator, the cytopathic effects (CPEs) were examined and scored as percentages of control untreated-infected wells. Each experiment included plant extract toxicity controls in uninfected monolayers in order to rule out toxic effects of the extracts. From concentration-effect curves, the 50% effective concentrations (EC50) were determined.

Table 2 indicates that aqueous extracts of GHX-2L, GHX-4L, and GHX-6L, had inhibitory activities against all viruses tested. GHX-7L, GHX-20L, and GHX-26F were tested against only HSV and showed selective activities against this virus. The concentrations at which the plants completely inhibited viral CPE, ie, EC100, were minimally toxic to Vero cells (see Fig. 3). There

were improved activities for the 90% methanol extracts of GHX-6L and GHX-20L over the aqueous extracts (compare tables 2 and 3). With respect to anti-HCMV activities tested in human embryonic lung (HEL) cells, only the roots of GHX-2 showed activity (Fig. 1).

Fig. 2 shows that variations in the time of initiation of GHX-2L and GHX-2R treatment post virus infection did not alter their activities. GHX-6L on the other hand was more effective when treatment was started early after virus infection. GHX-6L may therefore be acting primarily on early viral events whereas GHX-2L may be acting on late viral events. The significance of example 1 is made much more clear by example 3 (see below).

## 2. Effects of plant extracts on HSV infectious particles yield.

Having observed the antiviral activities by the effects of GHX extracts on cytopathicity, it was important to prove that viral replication itself was inhibited. This was done by determining the effects of the extracts on virus production (infectious virus particles yield). The procedure was the same as described in example 1 above except that at the end of the 3 days incubation, the cultures were freeze-thawed and titred for virus yield by the limiting dilution method (Ayisi et al, 1985). There were concentration dependent decreases in virus yields and this is exemplified by two plants in Table 3. This illustrates that the reductions in cytopathicity observed in example 1 are likely due to inhibitory effects of the extracts on viral replication.

## 3. Cytotoxicity of plant extracts in Vero cells.

The procedure for plant extract toxicity controls performed in example 1 is not very sensitive to detect cell survival. The tetrazolium-based colorimetry was therefore performed to confirm results of the cytotoxicity studies obtained in example 1. Uninfected monolayer Vero cultures were exposed to various

concentrations of plant extracts. After 3 days of incubation, the cultures were trypsinized and resuspended in maintenance medium. Percent cell survival compared to the control untreated cultures were determined by the tetrazolium metabolism method (Ayisi et al, 1991). The optical density (OD) values from treated wells without cells were subtracted from OD values of treated wells with cells. The resultant values were expressed as percentages of control untreated wells with cells (percent cell survival).

Fig. 3 shows the effects of various aqueous extracts of plants on Vero cell monolayers. 50% toxicity was not attained at any of the concentrations tested except in the case of GHX-7L where the very high concentration of 12.28 mg/ml caused 58% toxicity. The 50% effective concentrations (EC50s) of the seven plant extracts given in Table 2, did not cause any cytotoxicities. Likewise, the 100% effective concentrations (EC100s) of the seven plant extracts caused no or minimal cytotoxicities. The plant extracts are therefore selective and true antivirals.

4. Inhibitory effects of plant extracts against cytopathic effects of HIV-1, strains HTLV-IIIB and GH3 acute infections.

Having established the plant extracts as antivirals, especially against viruses like HCMV and HSV which are important opportunistic infections in immunosuppressed states, it was decided to test the extracts against a causative agent of immunosuppression like HIV. One of the modes of infection by HIV is acute infection whereby syncytia formation is induced by the virus leading to the death of several cells.

Example 4 demonstrates the activities of the plant extracts in HIV acutely infected cells. The determination of anti-HIV activities were similar to a previously published procedure (Ayisi et al, 1991). Briefly, 50 ul of virus or 50 ul of growth medium (plant extract toxicity controls) were added to respective wells of poly-l-lysine coated 96-well microtitre plates. 50 ul of Molt